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Effectiveness of Curcumin Supplementation in feed on liver productivity and reproductive performance of female carp *Cyprinus carpio* L.

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Abstract

The availability of quality seeds, on time with large amounts is a major problem in freshwater aquaculture. Supplementation of curcumin in female carp aims to increase the reproductive productivity of fish through increased activity of the liver in the synthesis of vitellogenin, a precursor of egg yolk, that play a role in the growth and development of eggs until larvae. This study used 25 female carp, which supplemented with curcuminat several doses: 0; 2.5; 5; 10; and 20 g of curcumin/kg of feed. The collected data was analyzed using a completely randomized design (CRD). The results showed that supplementation of curcuminin female carp significantly ($p<0.05$) affected the malondialdehyde(MDA) concentration of liver, estradiol hormone in blood, gonadosomatic index, relative fecundity, and larval survival, but did not significantly ($p>0.05$) affect the superoxide dismutase (SOD), vitellogenin concentration in blood and egg diameter.The study showed that the supplementation of curcumincan prevent the liver damage showed by a decreased level of liver MDA. The supplementation of curcumin with a dose of 5 g/kg of feed optimizes the productivity and reproductive performance of carp by increasing the production of vitellogenin, the diameter of egg, and the number of survival larva through optimizing the performance of the fish liver.

Keyword **:** *Cyprinuscarpio*L, curcumin, relative fecundity, survival rate of larvae,malondialdehyde(MDA)

INTRODUCTION

The synthesis of vitellogenin is an important part in the process of reproduction in fish. Vitellogenin is an egg yolk precursor synthesized by liver hepatocyte cells. The process of vitellogenesis begins with

the presence of the hormone estradiol which binds to the receptor cells of liver hepatocytes so that it stimulates the liver to synthesize vitelogenin. The resulting vitelogeninis then released into the plasma to the developing oocyte. This vetellogeninfurther become yolk, which used of embryo and larvae for growing and developing.

Carp is a freshwater fish that has a high fecundity the average of 177,786 eggs / kg body weight. High fecundity is thought to require more of egg yolk precursors to fulfill the demands of each growing oocytes. The number of oocytes that are developing simultaneously requires a supply of vitelogenin at the same time, which can cause disruption of liver function as a site for the synthesis of vitelogenin because liver cells must work harder. This situation weakens the physiological activity of the organ, thereby accelerating the occurrence of degeneration of liver cells, and followed by a decrease in the production of vitellogenin. This causes the lack of egg yolk that deposited in the oocytes thereby reducing the energy source for the development of embryos and larvae. Deficiency of egg yolk material will disrupt the development of embryos and larvae and can eventually cause death. Improvement of nutrition and physiological function in the broodstock during the process of vitellogenesis is one way that can support the adequacy of nutrients for the development of each egg formed (Izquierdo et al. 2001).

Curcumin is a phenol compound that has antioxidant activity and acts as a hepatoprotective (Anand et al, 2008; Sharma et al. 2015). Besides curcumin also has activity as phytoestrogens which can stimulate estradiol receptors (Bachmeier et al. 2010). Curcumin has a debilitating activity of inflammation and induces damaged cells to carry out apoptosis thereby avoiding hepatic fibrosis (Mu-En Wang et al. 2012).

Various studies have been conducted to determine the effects of curcumin in fish. In rainbow trout (Oncorhynchusmykiss) significantly increased the hematocrit value, erythrocyte count, and leukocytes (Yonar et al. 2013). In betook fish, Anabas testudineus (Bloch) showed an increase in antioxidant status against hepatocyte of hepatopancreas and an increase in glutathione content (Manju et al. 2012). In JuvenilLabeorohita (H), intraperitoneal injection of curcumin increases several non-specific immune system parameters (Behera et al. 2011).

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MATERIALS AND METHODS

2.1 Materials

The experimental fish were female carp (*Cyprinuscarpio*L) brood-stock taken from Freshwater Aquaculture Center,Tatelu. A total of 25 female carp were reared in five nets with size 2x2x1.5 m^3 and each net contained five fishes. The experimental fish used in this study have an initial body weight ranged of 2-2.5Kg. Acclimation was done for a week.

2.2 Food Material

The experimental feed was given by mixed the commercial ration (33 % protein) with curcumin according to the dose of treatment. The curcumin used in this experiment was produced by Plamed Green Science Limited with 93.71 % concentration of curcumin. The process of feed coating was started with the addition of carboxymethyl cellulose (CMC) powder as a binder to the commercial feed. The addition of CMC was 10% of the commercial feed used. Further, the curcumin was added into the commercial feed mixed with CMC. Feeding treatment was carried out for 40days of fish rearing.

2.3 Experimental design

The experimental design used was a completely randomized design with 5 treatments, each of which had 5 replications (each individual is a replication) as follows:

A = 0 g curcumin/kg feed (control)

B = 2.5 g curcumin/kg feed

C = 5 g curcumin/kg feed

D = 10 g curcumin/kg feed

E = 20 g curcumin/kg feed

1,2,3,4,5 = treatment replication

2.4 Sample collection

Before blood sampling and surgery, the fish were anesthetized. The blood sample was collected at the 20 and 40 days after treatment. The collected blood was put into a polyethylene tube and centrifuge at 3000 rpm for 10 minutes at 4 ˚C to obtain the serum. Serum was used to analyze the concentration of

estradiol hormone and vitellogenin. At the end of the observation the measurement of fish body weight was carried out before surgery. The gonads and livers obtained from surgery were weighed. The liver was rapidly excised and divided into two parts: the first part was kept in sterile Eppendorf tube, immediately was immersed in liquid nitrogen and then kept in -20˚C for Malone-dialdehyde determination (MDA) and Superoxide dismutase (SOD).Spawning was conducted naturally. After spawning the eggs diameter were measured by taking 200 eggs.About 100 larvae of each group of treatment and their replicate were taken from the hatching pool on their first day after hatching. The rearing of the larva was conducted for seven days without aeration and food.

2.5 Parameters Measurement

- Malondialdehyde (MDA) assay

  As much as 1 g of catfish liver stored frozen was then finely chopped under cold condition and dissolved in 2 ml of phosphate buffer saline (PBS) KCl at a pH = 7.4. The mixture formed was centrifuged at 10.000 rpm for 20 minutes and then the supernatant was taken for further MDA assay. The MDA concentrations of the liver to determine the peroxidation activity of liver cell membranes were measured by using the TBA method (Singh *et al. 2002*). TEP (1.1.3.3-Tetraethoxy-propabe, ≥96%) MW 220.31 (ALDRICH, USA) was used as a standard for MDA.

- Superoxide Dismutase (SOD) assay

  The determination of sample SOD activity was carried out according to Misra and Fridovich (1972). The principle of this method is based on the ability of SOD to inhibit epinephrine autoxidation to adenochrome. As much as 1 gram of fresh liver, put in a tube and added 2 mL PBS, homogenized and then centrifuged at 10000 rpm for 20 minutes. The obtained supernatant was transferred into a new tube as a sample for further analysis. Epinephrine 0.003 M solution was prepared by dissolving 5,496 mg of epinephrine with 10 mL HCL 0.01N. Measurements by spectrophotometer were carried out by adding 2,800 µL of 0.05M sodium carbonate buffer, 100 µL of the sample, and 100 µL of epinephrine solution into the cuvette and measured with wavelength absorption of 480 nm.

- Estradiol Assay

  Estradiol concentrations in the serum were determined with an enzyme linked immunosorbent assay (ELISA) method by using the kit estradiol (DRG Instruments GmbH, Germany).

- Vitellogenin assay

  Vitellogenin concentrations in the serum were determined with an enzyme linked immunosorbent assay (ELISA) method by using the Vitellogenin Fish Kit (KORAIN BIOTECH CO.LTD, CHINA).

- Gonadosomatic Index (GSI)

  Gonadosomatic index was measured by using the following formula:

$$IGS = \frac{\text{liver weight}}{\text{body weight}} \times 100$$

- Determination of relative fecundity

  Total number of ovulated eggs were divided by a total weight of broodstock gave the ratio of egg produced per gram body weight. The formula is given below:

$$\text{Relative fecundity} = \frac{Total\ number\ of\ ovulated\ eggs}{\text{Weight of Broodstock}}$$

- Eggs diameter

  Eggs diameter were measured using Zeiss microscopy then grouped according to the size of those egg diameter.

2.5 Data analysis

The data obtained were analyzed by using analysis of variance (ANOVA) on MINITAB version 16 program. The differences between the means of the treatment were tested by using Tukey simultaneous test. All results of significantly different were expressed with $p<0.05$.

RESULTS AND DISCUSSION

3.1 Effect of Curcumin supplementation on the liver of experimental fish on the reproductive period

The MDA concentration of liver fish after curcumin supplementation presented in Table 1 showed the significant difference ($p<0.05$) between treatments. The Group C showed the lowest level of MDA concentration (26.32±8.07 µg/g sample) followed by Group E (33.48±3.09 µg/g sample), B (42.22±16.97

µg/g sample), D (44.76±18.76 µg/g sample) and A (68.07±0.35µg/g sample), respectively. The SOD concentration aftercurcumin supplementation showed no significant difference (p>0.05) between treatments (Table 1).

Table 1. Concentration of MDA and SOD in the experimental fish liveron the reproductive period.

Parameters	Groups				
	A	B	C	D	E
Concentration of liver MDA(µg/g sample)	68.07±0.35[a]	42.22±16.97[b]	26.32±8.07[b]	44.76±18.76[b]	33.48±3.09[b]
Concentration of liver SOD(µg/g sample)	8.19±2.78[a]	10.44±3.69[a]	8.92±1.99[a]	7.99±2.15[a]	9.71±3.43[a]

Different superscripts in the same row and column indicate a significant difference

3.2 Effect of Curcumin supplementation to the concentration of estradiol hormone and vitellogenin of the experimental fish on the reproductive period

The average of the estradiol hormone concentration of the experimental fish in the pre-treatment was 118.00±8.33pg/ml. After 20 days of treatment, the concentration of estradiol hormone in serum showed significantly different (p<0.05) between treatments (Table 2). The highest concentration of estradiol hormone was showed by Group C (2232±34 pg/ml) and followed by Group B (1740±121 pg/ml), E (1455±58 pg/ml), A (1290±119 pg/ml) and D (901±59 pg/ml), respectively. The concentration of estradiol hormone on 40 days of treatment showedsignificantly different (p<0.05) between treatments. The Group A showed the highest concentration of estradiol hormone (2592±371pg/ml) followed by Group E (2082±193pg/ml), B (1898±140pg/ml), D (1659±176pg/ml) and C (1136±49pg/ml), respectively. Meanwhile, the concentration of vitellogenin in serum at 20 days of treatment showed no significantly different (p>0.05) between treatments.Nevertheless, the Group C and D showed a higher level of vitellogenin concentration than others (Table 2).

Table 2. Concentration of estradiol hormone and vitellogenin in serum of experiment fish on the reproductive period

Parameters	Groups				
	A	B	C	D	E
Estadiol hormone concentration at 20th days (pg/ml)	1290±119^{c}	1740±121^{b}	2232±34^{a}	901±59^{d}	1455±58^{c}
Estadiol hormone concentration at 40th days (pg/ml)	2592±371^{a}	1898±140^{b}	1136±49^{c}	1659±176^{bc}	2082±193^{ab}
Vitelogeninconcentration at 20th days (mg/ml)	5.70±1.23^{a}	5.66±0.23^{a}	8.12±1.81^{a}	7.09±1.02^{a}	5.65±0.61^{a}

Different superscripts in the same row and column indicate a significant difference

3.3 Effect of curcumin supplementation on reproductive performance of experimental fish

Gonadosomatic index presented in Table 3, showed significantly different ($p<0.05$) between treatments at the end of the study. Group A showed the highest of gonadosomatic index (21.86±0.73 %) followed by Group E (20.33±3.59 %), D (15.39±1.09 %), B (14.55±1.41 %) and C (14.46 ±3.28 %), respectively. The observation of the number of fecundity showed significantly different ($p<0.05$) between treatments.The highest number of fecundity showed by Group A (120,523±23,05 eggs/Kg BW), and followed by Group B (97,36±5,78 eggs/Kg BW), Group D (86,97±19,48 eggs/Kg BW), Group E (78,62±14,72 eggs/Kg BW) and Group C (55,833±10,79 eggs/Kg BW), respectively. Meanwhile, the diameter of eggs showed no significantly different ($p>0.05$) between treatments.The survival rate of larva at the seventh days after challenge showed significantly difference ($p<0.05$) between treatments. Group C showed the highest number of survavil larva (37,78±0.39 %) followed by Group A (31.78±0.19 %), D (31.45±1.35 %), E (29.89±1.35 %) and B (25.89±3.37 %), respectively.

Tabel 3. Gonadosomatic Index, fecundity, eggs diameterandsurvival rate of larvae

Parameter Pengamatan	Groups				
	A	B	C	D	E
Gonadosomatic Index (%)	21.86±0.73[a]	14.55±1.41[b]	14.46 ±3.28[b]	15.39±1.09[b]	20.33±3.59[ab]
Fecundity (the number of eggs/kg of body weight)	120,523±23,05[a]	97,36±5,78[ab]	55,833±10,79[b]	86,97±19,48[ab]	78,62±14,72[ab]
Diameter of spawning eggs (mm)	1.56 ± 0.10[a]	1.61 ± 0.10[a]	1.66 ± 0.11[a]	1.62 ± 0.10[a]	1.60 ±0.12[a]
Larval survival rate at seventh days of challenge (%)	31.78±0.19[b]	25.89±3.37[c]	37,78±0.39[a]	31.45±1.35[b]	29.89±1.35[bc]

Different superscripts in the same row and column indicate a significant difference

1. Discussion

MDA is a compound produced due to damage to the cell membrane. Damage to the cell membrane can occur by the presence of oxidant compounds. SOD itself is an endogenous antioxidant produced by cells to neutralize the oxidant compounds present in cells. These oxidant compounds can come from outside the cell or from inside the cell. Oxidant compounds originating from within the cell itself are a byproduct of the process of metabolism that occurs(Valko *et al.* 2007). In fish, during the process of reproduction the liver activity becomes very high because of its function as a place of synthesis of vitellogenin, a precursor of yolk. High liver activity due to the synthesis of vitellogenin and various other activities during the reproduction process allows for liver cells exposed to oxidant compounds(Kasiyati *et.al.* 2016a;Watson, 2002). Imbalance between oxidant compounds and antioxidants will cause

opportunities for damage to cells (Rahman, 2007).The results showed that supplementation of curcumin in feedhas reduced the MDAconcentration of fish liver.It means that curcumincan protect the liver from damage due to oxidant substance. This can be due to the function and activity of curcumin as a hepatoprotector.This activity of curcumin helps to maintain liver function from damage by playing a role both as an exogenous antioxidant and by influencing endogenous antioxidant activity via the activity of glutathione peroxidase (GTH) (Manju *et al.* 2012; Iqbal *et al.* 2003; Sharma *et al.* 2005). However, the value of endogenous antioxidants such as SOD did not differ between treatment groups. This can be due to the activity of curcumin, which only affects the endogenous antioxidant mechanism of GTH and does not affect the endogenous antioxidant mechanism of SOD.

The concentration of estradiol in blood serum can describe the condition of reproduction in fish that is taking place. The hormone estradiol is responsible for the process of vitellogenin synthesis. When the hormone estradiol binds to its receptors on hepatocyte cells, it will directly stimulate hepatocyte cells to start synthesizing vitellogenin.In this study, on the twentieth day after treatment the experimental fish in group C (concentration of 5 g curcumin/kg of feed) showed an increase in the concentration of the hormone estradiol in blood serum followed by an increase in the amount of vitellogenin detected in blood serum,butafter the fortieth day, there was a decrease in their hormone estradiol compared to other treatment groups which still continued to increase.From these results, it appears that the experimental fish in the Group Cexperienced faster gonad maturity compared to other treatments when viewed from the indicated estradiol hormone concentration. According to research conducted by Dewi (2018), the pattern of estradiol hormone concentration in fish will increase when at the peak of the synthesis of vitellogenin and will return down when entering the maturation stage.According to kasiyati et al. (2016b), the physiological state of the liver also has a major influence on vitellogenin synthesis. However, the supplementation of curcumin not only protects the liver cells but also increases their bioactivities as phytoestrogen that binds to the estradiol receptor and further stimulates the synthesis of vitellogenin (kasiyati et al., 2016a**).**This might be able to describe the condition of vitellogenin concentration in the Group D after twenty days of curcumin supplementation, which despite having lower levels of the hormone estradiol than control (Group A), but the concentration of vitelogenin in blood serum is higher.Meanwhile, in the treatment Group E with a higher concentration of curcumin(concentration of 20 g curcumin/kg of feed) and Group B with a lower concentration of curcumin (concentration of 2.5 g of curcumin/kg of feed), the vitellogenin concentration was no

different compared to control. It means that the dose of 5 and 10 g curcumin/kg of feed can optimize the synthesis of vitellogenin.Nevertheless, the results of the study showed no significant difference ($p>0.05$) between treatments of blood serum vitellogenin concentrations.It because the presence of vitellogenin in the blood circulation is influenced by the ability of hepatocyte cells to synthesize and secrete vitelogenin into the bloodstream and, the rate of absorption of the vitelogenin from the circulation to enter and deposited into the developing oocytes.According to cerda et al. (1996), the concentrations of vitellogenin in the plasma was basically constant throughout the reproductive cycle, except that there was more activity in taking vitellogenin by a growing population of eggs that decreased the level of vitellogenin in the plasma.

In this study, the gonadosomatic index values of all groups supplemented with curcumin showed the lower value than control. Therefore the relative fecundity also indicate that the control has a higher value.Even so, the size of the egg diameter of all treatment groups supplemented with curcumin was greater than the control.This shows that the administration of curcumin to female carp can increase the amount of vitelogenin deposited in the egg that appears through an increase in egg diameter, but the number of eggs decreases. This possibility is related to the mechanism of vitellogenin uptake by the growing egg, which not yet be able to explain in this study. The rearing of Larvae through challenge testing was conducted for seven days starting from the hatching. The results showed that the larvae produced from the group that supplemented with curcumin at a dose of 5 g/kg of feed have higher survival value. This may be related to the sufficient of yolk content for embryo growth and development to become larvae.The adequate availability of egg yolk under the larvae after hatching can determine its survival (Li and Mathias, 1987). That is because at the beginning of life larvae have not been able to get food from the external environment so that their lives depend entirely on the yolk underneath (Gisbert and Willot, 1997; Kamler 1992). Two days after hatching, the larvae are able to find their own food from external environment. Before able to get food from the external environment, they just only use yolk as the main source of energy. Adequacy of energy for larvae at the beginning of their life can also determine the vitality of these larvae so that these larvae have a better ability to live. Conversely, lack of energy for the beginning of development will cause larvae lack of ability to live.

2. Conclusion

The study showed that the supplementation of curcumin could prevent liver damage, shown by a decreased level of liver MDA. The supplementation of curcumin with a dose of 5 g/kg of feed optimizes the productivity and reproductive performance of carp by increasing the production of vitellogenin, the diameter of egg, and the number of survival larva through optimizing the performance of the fish liver and also can accelerate the maturation of gonadal. Nevertheless, the number of larval production is almost similar to control, because of the relative fecundity of control is higher.

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